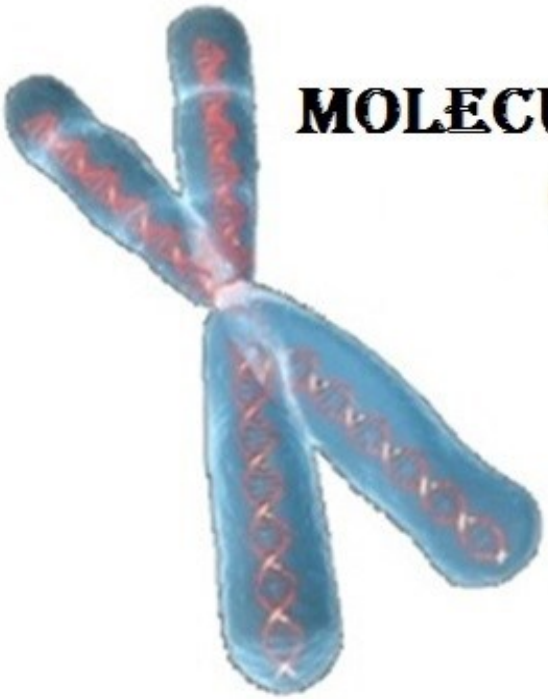
زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

Lab: 6

PRACTICAL

MOLECULAR GENETICS



Subject : RESTRICTION ENZYME

Department: Animal Resources

Stage : 2

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RESTRICTION ENZYME

Restriction enzymes are a special group of nucleases which make specific cut of DNA strands to produce fragments. They are a chemical knives used in engineering. They are discovered by Lederbreg and Meselson in 1964. These enzymes naturally occur in all bacteria. They are class of endonucleases, so they also called restriction endonucleases. 3,000 enzymes have been identified, around 200 have unique properties, and many are purified and available commercially.

Classification of restriction endonucleases:

Class	Abundance	Recognition site	Use in recombinant DNA technology
Type I	Less common	Cut both strands at a nonspecific location > 1000 bp away from recognition site	Not useful
Type II	More common	Cuts double strand at a specific, usually palindromic, recognition site 4-8 bp	Very useful
Type III	Rare	Cleaves single strand, 24- 26 bp downstream of the 3' recognition site.	Not useful

Nomenclature of Restriction Enzymes

They are named using the first letter of genus and the first two letters of species with strain abbreviation and numerical letter for strain containing more than one enzyme.

Example: EcoR1

Genus: **E**scherichia

Species: **co**li

Strain: **R**

Order discovered: **1**

Recognition Site:

Each restriction enzyme recognizes a specific base sequence about 4-8bp on the DNA strand this sequence is called **recognition site (palindromic)**. The ability of an enzyme to recognize a particular sequence is called **sequence specificity**.

A four-base cutter: Hpa II (*Haemophilus parainfluenzae*)

5'-C CGG-3'

3'-GGC C-5'

A six-base cutter: HindIII (*Haemophilus influenzae*)

5'-A AGCTT-3'

3'-TTCGA A-5'

An eight base cutter: Asc I (*Arthrobacter species - E. coli*)

5'- GG CGCGCC -3'

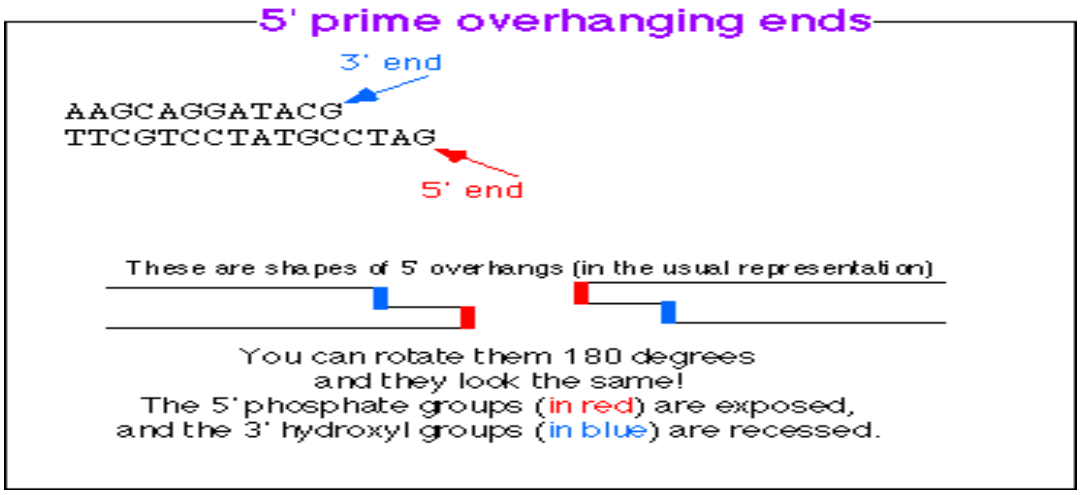
3'-CCGCGC GG-5'

Types of end Cut

Enzyme	Recognition sequence	Type of ends in product
BamHI	G^GATCC	5' overhang
SacI	GAGCT^C	3' overhang
SmaI	CCC^GGG	blunt

1. Sticky ends (cohesive ends):

a. 5’ overhang: BamHI (*Bacillus amyloliquefaciens)*

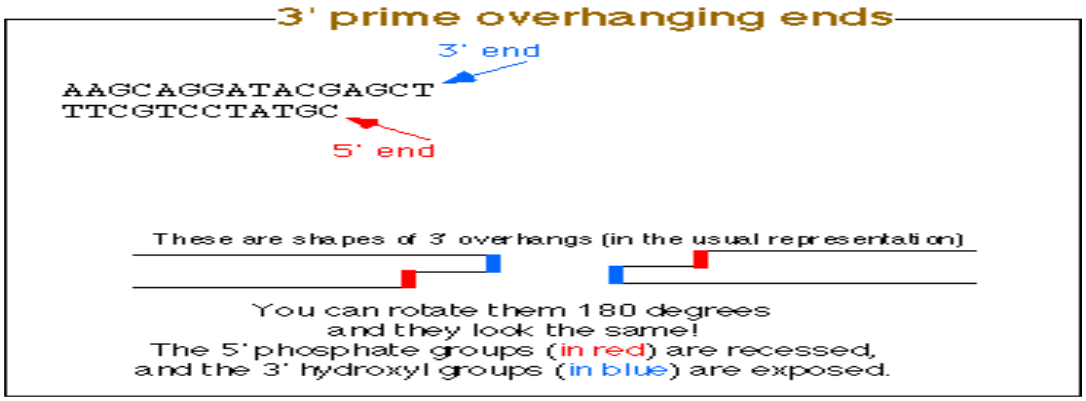


b. 3’ overhang:

For example: enzyme Sac I (*Streptomyces achromogenes)*

5'-GAGCT C-3'

3'-C TCGAG-5'

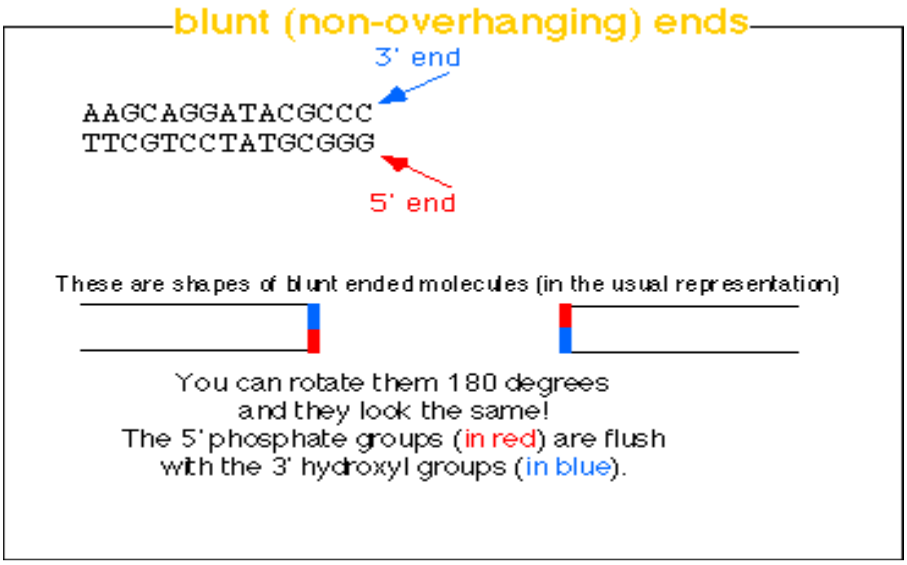


2. Blunt ends (neither end is overhanging) Flush (level):

For example, the enzyme Sma I (*Serratia marcescens)* cuts in the middle of the six nucleotide recognition sequence:

5'-CCC GGG-3'

3'-GGG CCC-5'



Example of use RFLP in the detection of mutation in disease state:

Mutation in the Methyl tetrahydrofolate reductase enzyme could lead to increase level of homocysteine in blood and leads to increase risk of thrombosis in these individuals carrying the mutation